Quantitative Trait Loci Associated with Seedling Resistance to Isolates of *Puccinia coronata* in Oat

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ABSTRACT

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In our previous report, quantitative trait loci (QTL) for field adult plant resistance to crown rust were identified in an oat population of 152 $F_{5:6}$ recombinant inbred lines from the cross of 'Ogle' (susceptible)/MAM17-5 (resistant). The objectives of the present study were to identify in the same population, the number, genomic location, and effect of QTL and digenic QTL epistasis associated with greenhouse seedling resistance to isolates of *Puccinia coronata* to determine if the QTL detected are isolate-specific and to compare them with previously detected QTL for field resistance. Reaction type was scored on greenhouse seedlings inoculated with three isolates. Composite interval mapping was conducted to identify genomic regions associated with resistance using a framework map of 272 molecular markers. Two QTL, *Pcq1* and *Pcq2*, were identified for resistance to each of the three isolates. *Pcq1*, the major QTL controlling field resistance, did not confer detectable greenhouse seedling resistance when present singly; however, *Pcq1* did serve as an enhancer of seedling resistance when it was combined with *Pcq2*. The final model explained 76.5, 77.9, and 79.3% of total phenotypic variation for resistance to isolates MNB248, MNB249, and MNB251, respectively. Race-specificity of quantitative resistance remains to be further examined.

Crown rust, caused by the fungus *Puccinia coronata* Corda f. sp. *avenae* Eriksson, is an economically important disease of oat (*Avena sativa* L.) and occurs in virtually all oat-growing regions of the world (34). The disease caused an average yield loss of 1 to 7% throughout the United States in the 1990s; however, in the early 90s, severe epidemics reduced total yields by as much as 20% in the north-central States, the major oat producing area in the United States (21). In recent years, crown rust has not caused widespread losses partly due to the use of resistant cultivars. Breeding for crown rust resistance has been almost exclusively through the utilization of monogenic resistance. Most of the resistance genes in recently released oat cultivars have been introgressed from the hexaploid wild oat species *A. sterilis* L. (22). *P. coronata* is highly variable in virulence and can rapidly evolve new pathotypes through mutation, hyphal anastomosis, or sexual reproduction on the alternate host, *Rhamnus* spp. (34). The development of new virulent pathotypes often renders these oat cultivars susceptible and results in the need for oat breeders and geneticists to identify and characterize new crown rust resistance sources.

Accessions of the diploid oat species *A. strigosa* L. were identified that are highly resistant to crown rust (26,36,41). A locus, *Pca*, conferring resistance to at least nine isolates of *P. coronata* in the accession CI 3815 of this species was mapped to linkage group A of the diploid map (28). Five genes, *Pc81*, *Pc82*, *Pc83*, *Pc84*, and *Pc85* were later assigned to this locus (43). The transfer of genes from the diploid *A. strigosa* to the hexaploid *A. sativa* (14) has been difficult; however, a few efforts have been successful. Resistance to race 264 and 294 in the accession CD 3820 of *A. strigosa* was incorporated into a number of hexaploid lines (11). Resistance in the *A. strigosa* accessions CD3820 and CI3436 was transferred to *A. sativa* selection MAM17-5 using a series of bridging crosses and irradiation of monosomic alien substitution lines (13,24,33). Crown rust resistance in hexaploid germ plasm Obee/Midsouth was thought to have come from one (or both) of the *A. strigosa* accessions CI4639 and CI3815 (31).

More than 100 crown rust resistance genes have been identified and 96 of them designated as *Pc* genes (7,22,30,35,43). In addition to the *Pc* genes, eight dominant inhibitor genes that counteract the effect of certain *Pc* genes have been identified (35,40,42). Most of the *Pc* genes are dominant, but some are partially dominant and others recessive (35). Resistance also can be controlled by two or more minor genes acting in an additive fashion (17). Some progress has been made in elucidating the placement and organization of these *Pc* genes in the hexaploid oat genome (2,3,30). Close linkage versus allelic relationships among these *Pc* genes however, has been difficult to establish.

Van der Plank (38) classified the plant-pathogen interaction systems into two general categories. One is race-specific and displays a hypersensitive reaction to pathogen infection. This is often called "vertical" resistance. The other type of resistance is race-nonspecific, quantitative in nature and often termed "horizontal" resistance. Some studies have determined that quantitative resistance might also be isolate- or race-specific (4,9,20,27); however, to our knowledge most quantitative trait loci (QTL) showing isolate specificity were minor ones, and only one major QTL reported in these studies (9) was clearly specific to pathogen races or isolates. Identification of QTL for disease resistance in oat has been limited (2,18,46,48). Recently, up to four QTL for field adult plant resistance to crown rust were identified in an oat mapping population of recombinant inbred lines (RIL) derived from the cross, 'Ogle'/MAM17-5 (46). Two major QTL, *Pcq1* and *Pcq2*, explained 48.5 to 70.1% and 9.6 to 14.0%, respectively, of the

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total phenotypic variation for crown rust resistance; however, the issue of the race-specificity was not addressed in the study.

Information regarding the number, genomic location, and effect of disease resistance genes and interactions between the genes would facilitate plant breeding for disease resistance. Determining race specificity of QTL would be helpful in understanding the concept of quantitative resistance. Finally, mapping crown rust resistance genes should be useful to gain a better understanding of the organization of rust resistance genes in the hexaploid oat genome. This study was undertaken, therefore, (i) to identify the number, linkage map position, and effect of QTL and digenic QTL epistasis associated with greenhouse seedling resistance to isolates of *P. coronata* in MAM17-5; (ii) to determine if the QTL detected are isolate-specific; and (iii) to analyze the linkage relationships of QTL associated with greenhouse seedling resistance and QTL controlling field adult plant resistance reported in the previous study (46).

MATERIALS AND METHODS

Plant materials. A mapping population of 152 $F_{5:6}$ RILs used in this experiment was derived from a cross between two hexaploid cultivated oat (*A. sativa* L.) genotypes, 'Ogle' (CI9401) and MAM17-5, with contrasting responses to the crown rust pathogen (*P. coronata*). MAM17-5 was selected in the spring oat breeding program at the University of Wisconsin—Madison (24), and is a crown rust resistant line with its resistance genes believed to originate from accessions CI3436 and CD3820 of the diploid oat *A. strigosa* L. (10). 'Ogle' was developed in the spring oat breeding program at the University of Illinois (1), and is a well-known oat cultivar lacking current crown rust resistance. The mapping population of RILs was developed using the single-seed-descent method.

Isolates of *P. coronata*. Isolates of *P. coronata* were collected from the University of Minnesota buckthorn (*Rhamnus cathartica* L.) nursery in 1998. The parents, 'Ogle' and MAM17-5 of the mapping population were screened first with seven of the collected isolates to determine the specificity of each isolate and all of them were avirulent on MAM17-5. Three isolates MNB248, MNB249, and MNB251, each giving a differential reaction between 'Ogle' and MAM17-5, were chosen to inoculate seedlings of the 152 RILs. These three isolates are maintained in the USDA-ARS, Cereal Disease Laboratory, St. Paul, MN. Their reactions have been determined on other oat lines, including 16 differentials (8) carrying 28 *Pc* genes (Table 1).

Crown rust evaluations. The seedling tests were conducted in the USDA-ARS, Cereal Disease Laboratory, St. Paul, MN in the winter of 1999 and repeated in late fall of 2000. Seedlings of the 152 RILs, the two parents 'Ogle' and MAM17-5, and the susceptible cv. Starter were grown under greenhouse conditions at 24/21°C, 12 h light/12 h dark. A randomized complete block design was used with 10 seedlings per experimental unit and two replications. Seedlings with first leaves fully expanded (approximately 1 week old) were inoculated with an inoculator containing fresh crown rust urediospores suspended in light mineral oil Soltrol 170 (Phillips Petroleum Co. Itex Plant, Borger, TX) at a concentration of approximately 1 million spores per ml. After 20 to 30 min to allow the oil to evaporate, the plants were placed into a dew chamber at 18°C with 100% humidity for 18 to 20 h. The plants were then moved back into the greenhouse for rust development. When full symptoms were clearly seen on the susceptible controls, 'Starter' and 'Ogle' (about 12 days after inoculation), seedlings were scored for their reaction type to *P. coronata*. If the 10 seedlings in an experimental unit displayed nonuniform reaction types, the entry was recorded as segregating and treated as missing data in the later linkage analysis. The modified method of Murphy (25) was used and is based on a 1 to 5 scale. On this scale: 1 = little or no visible reaction; 2 = no visible sporulation, but abundant flecks or necrotic spots; 3 = sparse small sporulating pustules and surrounded by prominent chlorotic spots; 4 = many small sporulating pustules and often surrounded by chlorotic areas; and 5 = many medium to large pustules and generally with little or no chlorosis.

Molecular marker analysis and map construction. Sources of restriction fragment length polymorphism (RFLP) clones, microsatellite or simple sequence repeat (SSR) primers, and amplified fragment length polymorphism (AFLP) primers were reported elsewhere (47). AFLP analysis was performed according to the protocol provided by the manufacturer (Gibco-BRL Life Technology, Inc., Gaithersburg, MD) with minor modifications (47). SSR analysis was performed according to Chin et al. (6), except that the separation and detection of the amplified products were done on polyacrylamide sequencing gels. RFLP analysis followed a standard protocol (47). For identification of QTL, a framework linkage map with 272 molecular markers was developed using the most informative markers (45).

Data analysis. Analysis of variance was performed by using the General Linear Model Procedure (Proc GLM) of SAS (SAS Institute, Inc., Cary, NC) (32). Mean values across replications were calculated for each RIL. These values were used to determine phenotypic correlations in the RIL population and to conduct QTL analysis. Pearson correlation coefficients were calculated by using the Correlation Procedure (Proc Corr) of SAS. The significance of genotype-by-year interaction was examined.

The analysis of QTL was performed using PlabQTL (37). The identification of QTL was conducted using the same procedures as described in a previous study (46). All regions with logarithm of odds (LOD) >3.5, corresponding to an experiment-wise error rate of 0.05, from the QTL analysis were considered significant and included in the final model. Composite interval mapping (44) with the cov SELECT option was used for QTL detection. The QTL position, given as centimorgans from the top of a linkage group, was determined when the LOD score reached its maximum. A support interval with a LOD fall-off of 1.0 was given for each

TABLE 2. Mean reaction type (RT) to isolates of *Puccinia coronata* for the two parents, their F_1 and recombinant inbred lines (RILs)

	Parents			RILs	
Traits[a]	'Ogle'	MAM17-5	F_1	Mean ± SD	Range
RT_{I1}	5.0	3.0	4.0	4.0 ± 0.9	2.3-5.0
RT_{I2}	5.0	2.0	4.0	4.0 ± 1.0	2.0-5.0
RT_{I3}	5.0	2.0	3.0	3.8 ± 1.1	2.0-5.0

[a] RT_{I1}, $RT_{I2,}$ and RT_{I3} = reaction type to isolate MNB248, MNB249, and MNB251, respectively.

TABLE 1. Reaction patterns of the three isolates of *Puccinia coronata* on *Pc* genes

	Avirulent	Virulent
MNB248	*Pc*38, *Pc*39, *Pc*40, *Pc*45, *Pc*48, *Pc*50, *Pc*52, *Pc*53, *Pc*54, *Pc*55, *Pc*58, *Pc*62, *Pc*63, *Pc*64, *Pc*67, *Pc*68, *Pc*71	*Pc*14, *Pc*35, *Pc*36, *Pc*46, *Pc*51, *Pc*56, *Pc*57, *Pc*59, *Pc*60, *Pc*61, *Pc*70
MNB249	*Pc*38, *Pc*45, *Pc*51, *Pc*53, *Pc*54, *Pc*60, *Pc*61, *Pc*62, *Pc*63, *Pc*64, *Pc*67, *Pc*68, *Pc*71	*Pc*14, *Pc*35, *Pc*36, *Pc*39, *Pc*40, *Pc*46, *Pc*48, *Pc*50, *Pc*52, *Pc*55, *Pc*56, *Pc*57, *Pc*58, *Pc*59, *Pc*70
MNB251	*Pc*35, *Pc*38, *Pc*39, *Pc*45, *Pc*48, *Pc*50, *Pc*51, *Pc*52, *Pc*53, *Pc*54, *Pc*55, *Pc*58, *Pc*59, *Pc*60, *Pc*61, *Pc*62, *Pc*63, *Pc*64, *Pc*68, *Pc*70, *Pc*71	*Pc*14, *Pc*36, *Pc*40, *Pc*46, *Pc*56, *Pc*57, *Pc*67

QTL. QTL with an overlapping support interval are assumed to be the same QTL. The additive effect of a QTL was calculated by PlabQTL as (mean of the homozygous MAM17-5 class - mean of the homozygous Ogle class)/2. The additive-by-additive epistasis was estimated using the Model AA command.

Isolate-specific QTL are defined in this study as QTL for resistance to different isolates either located on different chromosomes or having nonoverlapped support intervals when they are located on the same chromosome. QTL for resistance to different isolates are not considered to be isolate-specific if they have overlapped support intervals, although they may have different LOD score peaks.

RESULTS

Assessment of resistance to each of the three isolates. No significant genotype × environment (G × E) interaction was identified for reaction types between scores from seedling tests in the greenhouse in 1999 and those in 2000; therefore, the average of the 2-year data was used for QTL mapping. Analysis of variance indicated that highly significant differences were present between the two parents and among the 152 RILs for resistance to each of the three isolates. Reaction type to each of the three isolates was consistently higher for 'Ogle' than for MAM17-5, corroborating that the two parents differed in genes controlling these traits. Reaction types for the 152 RILs showed a relatively continuous distribution with most of the individual lines falling between the two parental types (Table 2; Fig. 1). Results suggested that no clear transgression existed for reaction types in the current study, and that resistance could be treated as a quantitative trait. On the basis of parental and F_1 values, no dominance and partial dominance were identified for resistance to isolates MNB248, and MNB251, respectively, whereas susceptibility to isolate MNB249 was partially dominant.

A significant correlation was found between traits measured, including field adult plant resistance (Table 3). Traits in the field have been investigated in a previous study (46). The correlation coefficients between resistance to the isolates in the greenhouse were high with $r > 0.90$. The correlations between seedling resistance to the individual isolates and general field resistance were low with $r < 0.40$, since some individual lines were clearly resistant in the field but susceptible in the greenhouse and vice versa.

QTL for resistance to each of the three isolates. A total of two QTL were identified in this population for resistance to each of the three isolates MNB248, MNB249, and MNB251 (Fig. 2; Table 4). Two QTL for resistance to each of the three isolates were all designated as *Pcq1* and *Pcq2*, respectively, since the supporting intervals for QTL controlling resistance to the three isolates were overlapping one another. The major QTL, *Pcq2*, was located on linkage group 29 and tightly linked to marker bcd1280a. This QTL explained 56.3, 50.8, and 62.2% of the phenotypic variance for resistance to isolates MNB248, MNB249, and MNB251, respectively. This was the largest portion of the phenotypic variance explained by corresponding fitting models. The other QTL, *Pcq1*, was located on linkage group 28 and explained 11.3, 16.9, and 12.3% phenotypic variation for resistance to isolates MNB248, MNB249, and MNB251, respectively. Negative QTL effects indicated that resistance alleles were all contributed by MAM17-5, which was in agreement with the absence of clear transgression for the traits in the RILs.

Significant ($P < 0.01$) epistatic effects between *Pcq1* and *Pcq2* were found for resistance to each of the three isolates (Table 4). The QTL-by-QTL interaction was included in the model for the simultaneous fit, and the final model explained 76.5, 77.9, and 79.3% of total phenotypic variation for resistance to isolates MNB248, MNB249, and MNB251, respectively. The influence of *Pcq1*-by-*Pcq2* interaction on resistance to isolate MNB249 is demonstrated in Figure 3. RILs were classified into four classes based on the allele constitution of the closest marker loci for the two QTL. The RILs homozygous for the 'Ogle' allele at *Pcq2*

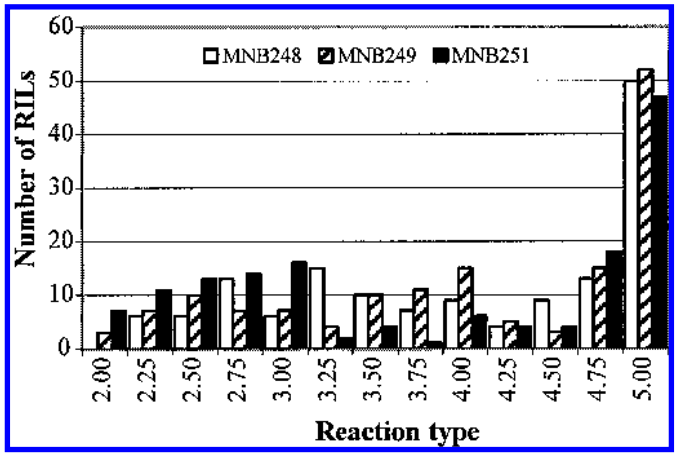

Fig. 1. Frequency distributions of reaction type to three isolates of *Puccinia coronata* in greenhouse seedling tests for the 152 recombinant inbred lines from the cross, 'Ogle'/MAM17-5. Reaction type was recorded based on a 1 to 5 scale in which 1 is highly resistant and 5 is highly susceptible. The values next to the x-axis are the upper limit of each category.

TABLE 3. Phenotypic correlation coefficients between the traits for data collected from the greenhouse and field

	RT_{99}[a]	RT_{00}	S_{99}	S_{00}	RT_{I1}	RT_{I2}
RT_{I1}	0.31**	0.32**	0.33**	0.35**		
RT_{I2}	0.35**	0.37**	0.36**	0.38**	0.94***	
RT_{I3}	0.24**	0.25**	0.26**	0.26**	0.95***	0.93***

[a] RT_{99} and RT_{00} = field reaction type in 1999 and 2000, respectively; S_{99} and S_{00} = field severity rating in 1999 and 2000, respectively (45). RT_{I1}, RT_{I2} and RT_{I3} = reaction type to isolate MNB248, MNB249, and MNB251, respectively. ** and *** = significant at the 0.01 and 0.001 levels of probability, respectively.

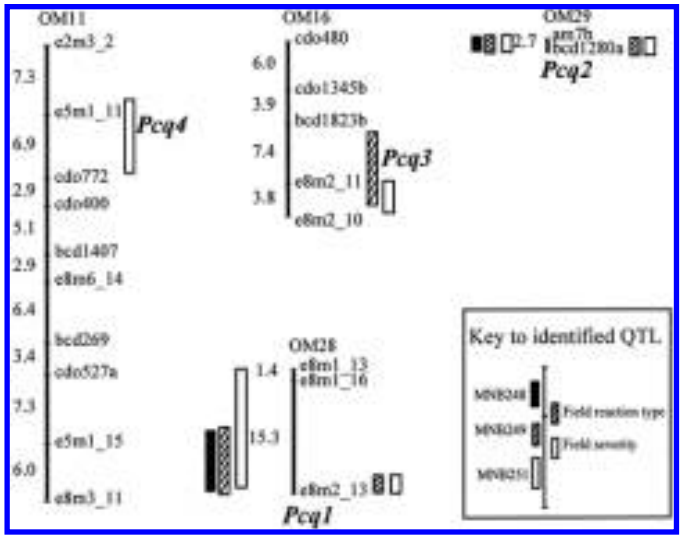

Fig. 2. Linkage groups from the map developed on the cross, 'Ogle'/MAM17-5 (OM), showing significant quantitative trait loci (QTL) for crown rust resistance. QTL for resistance to the isolates, MNB248 (solid bars), MNB249 (hatch bars), and MNB251 (open boxes) are indicated to the left of linkage groups. QTL for field resistance are indicated to the right of linkage groups by hatch bars (reaction type) and open boxes (severity). Seedling resistance to the three isolates is controlled by *Pcq1* and *Pcq2*. Field adult plant resistance based on reaction type is controlled by *Pcq1*, *Pcq2*, and *Pcq3*. Field adult plant resistance based on severity is controlled by *Pcq1*, *Pcq2*, *Pcq3*, and *Pcq4* (45).

displayed highest reaction types, typical of the susceptible parent 'Ogle', regardless of whether they were homozygous for the 'Ogle' allele or the MAM17-5 allele at *Pcq1*. However, the RILs homozygous for the MAM17-5 allele at both *Pcq1* and *Pcq2* showed greater resistance than the RILs homozygous for the MAM17-5 allele only at *Pcq2*. Similar results were found for the other two isolates. Thus, the MAM17-5 allele at *Pcq1* enhanced the expression of seedling resistance in the greenhouse for plants that also had the MAM17-5 allele at *Pcq2*.

Isolate specificity of QTL. Isolate specificity of QTL for crown rust resistance in the population has been investigated using three isolates to challenge the 152 RILs. If QTL for resistance are isolate-specific, we expect that a QTL for resistance to one isolate will not be identified for resistance to another isolate. From Table 4, however, two QTL, *Pcq1* and *Pcq2*, were consistently identified for resistance to each of the three isolates. The supporting intervals for QTL controlling resistance to the three isolates were overlapping one another on either linkage group 28 or 29, although QTL on linkage group 28 showed different LOD score peaks for resistance to the three isolates (Fig. 4). The reaction type of individual RILs to each of the three isolates was examined, and none of the RILs displayed significant reaction differences between rust isolates. We consider either one of the two genomic regions as a single QTL, because different QTL could not be confidently resolved. No clear isolate-specificity was, therefore, detected for the two QTL identified for resistance to the three isolates.

Relationship between QTL for resistance in the greenhouse and the field. Crown rust resistance in the field has been investigated in a previous study (46). Three QTL controlling reaction type and four QTL controlling rust severity in the field were putatively identified; however, only two QTL were consistently detected for the two traits in both years. The two QTL were located at the same positions as the two QTL identified in this study for resistance to each of the three isolates in the greenhouse. Therefore, we intentionally designated them as the same names, *Pcq1* for QTL on linkage group 28 and *Pcq2* for QTL on 29, for all the traits (Fig. 2); however, the major QTL for resistance to the three isolates in the greenhouse and resistance in the field are different. *Pcq1* contributed adult plant resistance in the field at least 5 times greater than it contributed seedling resistance to the isolates in the greenhouse, whereas *Pcq2* contributed seedling resistance to the isolates at least 5 times greater than adult plant resistance in the field (Table 4) (46).

DISCUSSION

Two QTL were identified that provide greenhouse seedling and field adult plant resistance, although field adult plant resistance was mainly controlled by *Pcq1* and greenhouse seedling resistance was mainly controlled by *Pcq2*. The bimodal-like distribution of the resistance traits in each case suggested that there might be only one QTL controlling the resistance. Moreover, the possibility exists that the two QTL could merge into one, since the two QTL were located on two small linkage groups, 28 and 29. The two validation analyses described in the previous research (46) supported that the two small linkage groups, 28 and 29 were not linked, and more than one QTL control the seedling resistance to each of the three isolates. Therefore, the conclusion can be made that two QTL control seedling resistance to each of the three isolates in oat line MAM17-5.

If we consider RILs with reaction type not greater than 3.5 to be resistant and greater than 3.5 to be susceptible, the RILs could be classified as either resistant or susceptible parental types. Classical inheritance analysis of disease resistance has been conducted in this way. Using the simplified data, only one major QTL was identified for resistance to the isolates, and only one major QTL for resistance in the field. Loci with smaller effects were not detected. On the basis of this result, classical linkage analyses were performed between resistance to the three isolates and field resistance to determine if both field and greenhouse resistance could be controlled by the same major QTL. Chi-square tests indicated that the ratio of RILs with recombinant type to parental type were not significantly different from a 1 to 1 ratio (Table 5). This analysis indicated that no linkage relationship existed between the resistance in the greenhouse and the resistance in the field. Therefore, the major QTL controlling greenhouse resistance and the major QTL controlling field resistance were different. The field readings most likely reflected the adult plant reactions, whereas the greenhouse tests represented only the seedling reactions. On

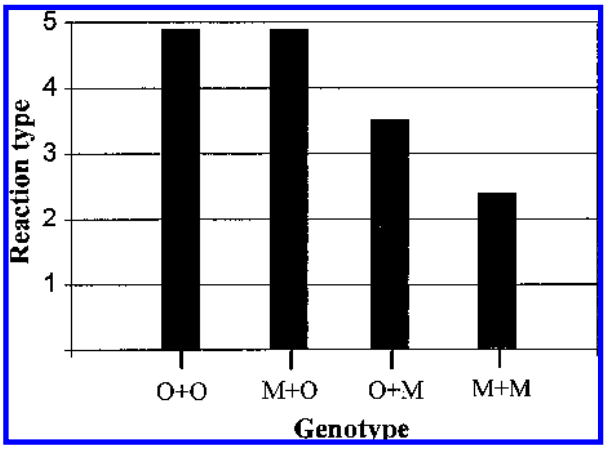

Fig. 3. Epistasis of two quantitative trait loci (QTL), *Pcq1* linked to marker e8m2-13 on linkage group 28 and *Pcq2* linked to bcd1280a on linkage group 29, for resistance to isolate MNB249. Letters O and M indicate the alleles of the corresponding QTL from 'Ogle' and MAM17-5, respectively. The symbol of genotype of recombinant inbred lines is *Pcq1* + *Pcq2*.

TABLE 4. Summary of quantitative trait loci (QTL) for resistance to the three isolates MNB248, MNB249, and MNB251 of *Puccinia coronata*. Reaction type data collected on 152 recombinant inbred lines derived from 'Ogle'/MAM17-5 were used. The QTL analysis was conducted using composite interval mapping with a logarithm of odds (LOD) threshold of 3.5

			MNB248			MNB249			MNB251		
QTL	Position[a]	Support interval	LOD	Exp.(%)[b]	Effect[c]	LOD	Exp. (%)	Effect	LOD	Exp.(%)	Effect
Pcq1 (1)	28:12	9-16	4.8	11.3	–0.22						
Pcq1 (1)	28:10	8-16				8.8	16.9	–0.31			
Pcq1 (1)	28:6	0-16							4.6	12.3	–0.25
Pcq2 (2)	29:2	0-2	52.2	56.3	–0.81	49.8	50.8	–0.84	55.9	62.2	–1.02
(1) × (2)				8.9	–0.19		10.2	–0.23		4.8	–0.15
Total[d] (%)				76.5			77.9			79.3	

[a] Linkage group and distance (centimorgans) from the top of the linkage group.
[b] Explained phenotypic variance obtained from the simultaneous fit of all putative QTL and significant QTL × QTL epistasis.
[c] Additive effect for QTL. Negative values indicate that MAM17-5 carries unfavorable (resistance) alleles for reaction type scores.
[d] The adjusted R^2 for the final model of simultaneous fit.

the other hand, rust pustules were randomly collected from Wisconsin field plots, and the virulence pattern of these field isolates on the differentials was compared with that of the three isolates used. None of the field isolates was the same as any of the three (data not shown). Therefore, the difference in the effect of the two QTL, *Pcq1* and *Pcq2*, on greenhouse and field resistance could result from either the difference in the traits measured or the difference in virulence composition represented by the three isolates in the greenhouse and the ones in the field.

Significant epistasis between *Pcq1* and *Pcq2* was identified on seedling resistance to each of the three isolates in the greenhouse. *Pcq1* was the major QTL controlling adult plant resistance in the field (46), and it did not confer detectable seedling resistance in the greenhouse when it was present singly, compared with *Pcq2*; however, *Pcq1* did serve as an enhancer of seedling resistance when it was combined with *Pcq2*. *Pcq1* enhanced the seedling resistance of *Pcq2* in a manner similar to that observed in genes for adult plant resistance to wheat leaf rust (15,19). In wheat leaf rust, adult plant resistance genes *Lr13* and *Lr34* did not lower leaf rust reaction types in the seedling stage by themselves; however, they enhanced seedling resistance when combined with genes for partial seedling resistance. The interactions between *Lr13* or *Lr34* and other genes were believed to confer the high level of durable resistance to leaf rust in wheat cultivars. Similarly, the interaction for enhanced resistance between *Pcq1* and *Pcq2* may contribute to the durability of crown rust resistance in oat line MAM17-5 since in several years of testing we have not identified any isolates from the Buckthorn Nursery in St. Paul, MN or mixed races in the field that were virulent on MAM17-5 (data not shown).

No clear isolate-specific QTL for the interaction system of *Avena sativa–Puccinia coronata* were identified in this study. Other research on the host-pathogen interaction systems between *Hordeum vulgare* and *Puccinia hordei* (27), *Capsicum annuum* and *Potyvirus* spp. (4), and *Solanum tuberosum* and *Phytophthora infestans* (20), however, indicated that some QTL for quantitative resistance were isolate-specific. Two minor QTL in our previous research were identified for field resistance to mixed races, which were different from the three isolates used in this study (data not shown), but these QTL did not contribute detectable resistance to the isolates in this study. It is of interest to notice that minor QTL often show isolate specificity, and major QTL often appear not to be isolate specific. Isolate-specific QTL for barley resistance to *P. hordei* were all minor QTL, and three major QTL were effective to both isolates used in the study (27). Similarly, minor QTL showed isolate specificity, and major QTL showed no specificity to pathogen races or isolates tested in other studies (2,4,5,20). Based on the hypothesis of Robertson (29), qualitative resistance may be an extreme form of quantitative resistance. If quantitative resistance is race-nonspecific, minor QTL with small effects should be identified for resistance to all isolates, and some major QTL with large enough effects should be isolate-specific. It has been difficult to prove this since QTL mapping, like any other genetic study, is only as good as its phenotypic scoring method. Minor QTL have only small effects on resistance, and correspondingly the relative proportion of environmental effect on these QTL is large. As a result, these minor QTL often have not been detected for different isolates. Thus, they appear isolate specific like what had been reported in most previous papers. These reports on minor QTL isolate specificity may not be completely reliable.

On the other hand, major QTL are easily detected across different environments or for different isolates, but if isolates were not chosen properly (for example, they do not belong to different races), results might appear to be race nonspecific, like most previous reports including ours. When the size of populations and the number of markers are limited, major QTL should be targeted to confirm if quantitative resistance is race (isolate)-specific, based on the assumption that results should be more reliable than those for minor QTL. Whether QTL identified in this study are isolate-specific remains to be further examined by either testing with a large number of isolates or fine-mapping the QTL detected. On the one hand, the three isolates of *P. coronata* were all collected from the University of Minnesota Buckthorn Nursery, and they may represent a small fraction of the pathogen spectrum. If more diverse isolates are tested, the QTL for crown rust resistance identified in this study may turn out to be isolate-specific. On the other hand, the QTL were located on either small fragments or sparsely marked regions of a linkage group. Therefore, it has been difficult to tell whether the same QTL has pleiotropic effects or whether tightly linked QTL are being mapped as the same locus. This could be determined by fine-mapping QTL through developing near isogenic lines and adding more markers to the specific QTL regions.

Resistance genes for the same or different pathogens are proposed to cluster in various genomic regions in plants (12,23). Marker bcd1280a cosegregated with isu2192a (data not shown), which was mapped to linkage group A of the diploid map (28). A locus, *Pca*, conferring resistance to at least nine isolates of *P. coronata* in the accession CI 3815 of *A. strigosa* was found to be linked to isu2192 (28,42). Five resistance genes, *Pc81*, *Pc82*, *Pc83*, *Pc84*, and *Pc85* were later assigned to this locus (43). Crown rust resistance in MAM17-5 was believed to be derived from accessions CI 3436 and/or CD3820 of *A. strigosa* (10). Therefore, the QTL, *Pcq2*, identified in this study and tightly linked to bcd1280a on linkage group 29, may belong to the same gene family as the *Pca* locus. Linkage group A of diploid oat has

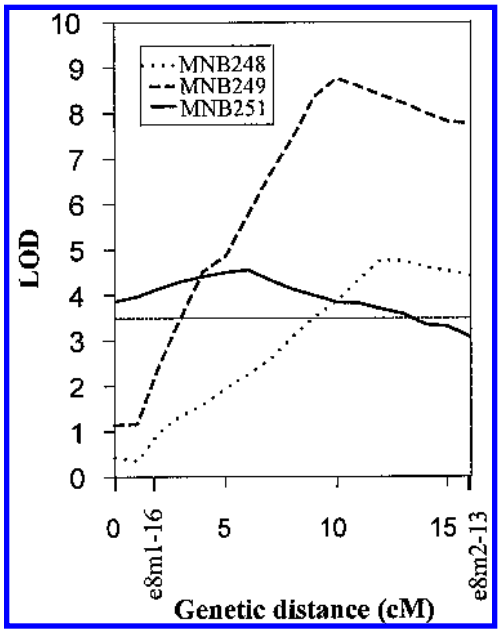

Fig. 4. Logarithm of odds (LOD) score profiles of quantitative trait loci (QTL) for resistance to isolates, MNB248 (dotted line), MNB249 (dashed line), and MNB251 (solid line) on linkage group 28 based on composite interval mapping. Linkage group 28 corresponds to OM28 in Figure 2 and is oriented with the top to the left. Thick tick marks on the x-axis indicate position of molecular markers. The closest marker to the significant QTL is shown below the x-axis. A threshold of LOD = 3.5 was used to define a QTL.

TABLE 5. Classical linkage analysis between reaction type to the three isolates in the greenhouse and reaction type in the field

	Recombinant	Parental	X^2	P
RT_{I1} versus RT[a]	66	84	2.16	0.25
RT_{I2} versus RT	65	84	2.42	0.25
RT_{I3} versus RT	71	76	0.17	0.75

[a] RT_{I1}, RT_{I2}, and RT_{I3} = reaction type to isolate MNB248, MNB249, and MNB251, respectively. RT = field reaction type (45).

been identified as corresponding to the homoeologous group 1 of Gramineae (39). A variety of disease resistance genes have been mapped on homoeologous group 1 in the Gramineae such as leaf rust (*Lr21*) and stem rust (*Sr33*) resistance in wheat (39), and powdery mildew (*Ml*) resistance in barley (16). All these results confirmed that disease resistance genes could be organized in clusters on a few chromosomes instead of random dispersion throughout whole plant genome.

Our evidence for lack of virulence to overcome the MAM17-5 resistance in existing field populations of *P. coronata* is limited to the isolates from the Buckthorn Nursery in St. Paul, MN that were tested on MAM17-5 in the greenhouse and to the observations of only limited crown rust development on MAM17-5 in field plots in Wisconsin and Minnesota (data not shown). Nevertheless, this evidence suggests that virulence to the MAM17-5 resistance, if it exits, is at least uncommon in the field at this time. Therefore, the QTL associated with the MAM17-5 resistance will be useful for incorporating MAM17-5 resistance into oat breeding lines with other effective resistance genes to reduce the chances of the combined resistance being overcome by a single virulence in the pathogen. Identification of molecular markers associated with the MAM17-5 resistance will facilitate selecting lines with combinations of resistance types. Phenotypic selection for crown rust resistance can be difficult given the complex interactions among the QTL (genes) of the host, races (isolates) of the pathogen, and environmental conditions. With marker-facilitated selection, it should be possible to select lines carrying the favorable alleles at all major loci without having to rely on the availability of rust cultures having the appropriate virulence. Additionally, the QTL can be transferred into other oat varieties relatively easily since the QTL detected in this study are carried in adapted, cultivated oat lines.

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